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Original Article

IN VITRO ACTIVITY OF VARIOUS POTENCIES OF HOMEOPATHIC DRUG *THUJA* AGAINST MOLDS INVOLVED IN MYCOTIC KERATITIS

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ABSTRACT

Objective: Isolation and characterisation of clinically isolated fungi from mycotic keratitis and exploration of the *in vitro* efficacy of various potencies of homeopathic preparations of *Thuja occidentalis* on the ocular fungal isolates.

Methods: Clinical samples were collected from fungal keratitis patients attending a tertiary eye care hospital in Coimbatore, Tamilnadu state, India. The scrapings were also subjected to Gram staining and 10% KOH mount to detect the presence of fungal hyphae. The fungal isolates were subjected to lacto phenol cotton blue (LCB) mount employing cello tape flag method. Homeopathic drug *Thuja occidentalis* with various potencies *viz*., Q, 30 C, 200 C, 1 M, 10 M and 50 M were investigated for the growth inhibition of various fungal isolates by plate assay method. Further, a follow up analyses with varying dilutions of Q and 10 M homeopathic potencies of *Thuja* was carried out for the determination of minimum inhibitory concentration of both microdilution and minimum fungicidal concentration for the *Biopolaris* isolates.

Results: Out of 35 samples analysed, *Fusarium* spp. (n=5), *Aspergillus flavus* (n=6), *Bipolaris* spp. (n=3), *Exserohilum* spp. (n=3) and *Curvularia* spp (n=3) were identified. All the potencies of *Thuja* had good inhibitory activity against *Bipolaris* spp., followed by *Curvularia* spp., *Exserohilum* spp. and *Aspergillus flavus.* Statistical analysis revealed significant inhibition of all the test isolates by *Thuja* Q and 50 M. Significant growth inhibition was exhibited by *Thuja* 30, 200 C, I M for *Bipolaris*, *Exserohilum* & *A. flavus* isolate and *Thuja* 10 M for all the isolates tested except Fusaria. It was revealed that for the *Biopolaris* isolates BS1, BS2, BS3 *Thuja* 10 M and *Thuja* Q had MIC and MFC of $0.125/10^{20000}$& 0.0625/10 and $0.25/10^{20000}$ & 0.125/10, respectively.

Conclusion: The present investigation concludes that homeopathic drug *Thuja* has good inhibitory activity against the fungi causing keratitis, irrespective of the potencies. It is evident that no definite co-relation exists between various potencies of the same homeopathic drug with regard to their antimycotic properties.

Keywords: Mycotic keratitis, Fungal isolates, Homeopathy, *Thuja occidentalis* potencies and Growth inhibition.

INTRODUCTION

Keratitis is an 'inflammation of the eye's cornea' that results from infection by microorganisms like bacteria, fungi, virus *etc*. Of these organisms, fungi are responsible for 30% to 40% of corneal infection [1]. In India, nearly 30-35% of all culture positive infectious keratitis are caused by fungi [2]. This infection is difficult to treat and it can lead to severe visual impairment or blindness. Mycotic keratitis has been reported from many different parts of the world, particularly tropical areas, where it may account for more than 50% of all ocular mycoses, or all cases of microbial keratitis [3]. More than 105 species of fungi, spanning 56 genera have been reported to cause oculomycosis [4]. From these, fungal genera of *Fusarium* spp. and *Aspergillus* spp. are responsible for 70% of cases. The other fungi that cause keratitis are *Curvularia* spp., *Alternaria* spp., *Bipolaris* spp., *Exserohilum* spp., *Candida* spp., and *Cladosporium* spp. The etiologic agents of mycotic keratitis show a varying pattern with respect to geographic locality and climatic conditions. In North India, the majority of mycotic keratitis were due to filamentous fungi, namely *Aspergillus* and *Fusarium* species. *Aspergillus* species were the most common isolate in fungal keratitis reported by Srinivasan *et al*. [5]. However, *Fusarium* species were found to be the most common cause of fungal keratitis from South India (Madurai and Tamilnadu) by Barathi *et al*. [1, 6] and Srinivasan *et al*. [5]. Symptoms of fungal keratitis are blurred vision, a red and painful eye, increased sensitivity to light (photophobia), and excessive tearing or discharge. Usually associated with fungal ulcer is hypopyon which is mostly white fluffy appearance. Rarely, it may extend to the posterior segment to cause endophthalmitis in later stages, leading to the destruction of the eye. Keratitis due to filamentous fungi is believed to usually occur following trauma, the key predisposing factor, in healthy young males engaged in agricultural or other outdoor work. The traumatising agents can be of plant or animal origin (even dust particles), that either directly implant fungal conidia in the corneal stroma or abrade the epithelium, permitting invasion by exogenous fungi [7]. Fungal keratitis if not diagnosed and treated properly, can rapidly destroy the integrity of the eye, resulting in ocular damage. Antifungal agents used for treatment include polyenes, azoles and fluorinated pyrimidines. Prolonged use of these antibiotics may cause undesirable side effects. There are reports of *in vitro* antifungal resistant organisms due to the frequent use of azoles, polyenes and 5-fluorocytosines.

Hence it is highly imperative to employ alternative antifungal agent with minimum / no side effects. Thus, the quest for effective antifungal agents which are clinically safe, non toxic, with minimal or no adverse systemic drug reactions, opens window to complementary and alternative therapies like homeopathy, due to use of ultra-high dilutions of a single natural substance as its medicines. Homeopathy medicines have been long used for treatment of human diseases of fungal origin. Also homeopathy claims to treat in ophthalmic problems ranging from cataract to conjunctivitis and the treatments remain largely free of potential harm to patients. One of the important homeopathic drugs which is used for the treatment of fungal infection is *Thuja occidentalis. Thuja*

is mainly used to treat eye problems such as eye inflammation, eye tumour; wart like growth in eyelid *etc*. This drug is obtained from *Thuja occidentalis*, an evergreen, native North American tree which grows to a mature height of 30 to 50 feet. *Thuja* contains an essential oil known as thujeine which has antimicrobial activity. It has been reported that *Thuja* 30 C and 200 C is very effective against *Aspergillus flavus* causing cutaneous aspergillosis in human and *Thuja* 50 M is effective against *Aspergillus niger* known to cause otomycosis [8]. In this context, the present study was carried out with the following objectives: isolation and characterisation of fungi from mycotic keratitis and exploration of the *in vitro* efficacy of various potencies of homeopathic preparation of *T. occidentalis* on the ocular fungal isolates.

MATERIALS AND METHODS

Collection and processing of samples

Clinical samples were collected from fungal keratitis patients attending a tertiary eye care hospital in Coimbatore, Tamilnadu state, India. The specimen *viz*., corneal scrapings and corneal swabs were collected from fungal keratitis patients by using a sterile Kimura's spatula aseptically under slit lamp illumination after administering a local anesthetic [9]. The scrapped out materials were inoculated directly into solid media such as blood agar and Sabouraud's dextrose agar (SDA). The scrapings were also subjected to Gram staining and 10% (KOH) mount to detect the presence of fungal hyphae [10].

Identification of fungal isolates

The fungal isolates obtained were point inoculated on the centre of SDA plates and incubated at room temperature for 3-4 days. The fungal isolates were subjected to lacto phenol cotton blue (LCB) mount employing cello tape flag method [11]. The preparation was examined microscopically under 10× and 45× for observing the details of spore body, spore morphology *etc*. [12]. Identified fungal isolates were subjected to single spore culture method as described by Leslie *et al*. [13]. All the isolates were maintained in 0.85% of saline solution in a screw capped storage vials and stored at room temperature for further studies.

Determination of antifungal activity of homeopathic drug *Thuja*

i) Plate assay

Homeopathic drug *Thuja* with various potencies *viz*., Q, 30 C, 200 C, 1 M, 10 M and 50 M were purchased from the manufacturer. Precisely, 0.5 ml of each potency of *Thuja* was mixed with 10 ml of sterile PDA at 45°C in 70 mm Petri dishes. PDA plates with 0.5 ml sterile water, 0.5 ml rectified spirit and 0.5 ml of 5 mg ketoconazole was used as growth control, spirit control and negative control, respectively. All the plates were aseptically inoculated with 1.5 mm diameter mycelial disc of test fungal isolates from 8 to 10 days old PDA culture plate. Plates were incubated at room temperature for five days. The radial diameter of the fungal growth was measured on third, fourth and fifth days of incubation. The percentage growth inhibition was calculated as follows:

Percent growth inhibition = $\frac{dc-dt}{dc} \times 100$, where dc is mean radial growth diameter of the control test isolate in growth control plate and dt is the mean radial growth diameter of the isolates in the treated plates.

ii) Broth microdilution assay

Using a sterile moistened swab, the spores were harvested from 7 days old PDA culture and then introduced into a tube containing sterile distilled water. Tween 20 (approximately 0.01 ml) was added to facilitate better spore suspension. The optical density (OD) of the spore suspension was adjusted using sterile distilled water to 0.15 – 0.18 at 530 nm which corresponded to the concentration of 0.5 x 10^6 to 4 x 10^6 CFU/ml. The suspension was then diluted with sterile water in the ratio 1:50 to get the final concentration of 0.4× 10^4 to 5× 10^4.The drugs chosen for the study were *Thuja occidentalis* Q and *Thuja occidentalis* 10M. The procedure was carried as per the CLSI [14] guidelines with slight modifications. For each homeopathic drug, 2 ml was taken as stock in a test tube (T_1). A total of 9 test tubes were taken for dilution series and marked as T_2-T_{10}, respectively. Precisely 1 ml of sterile RPMI-1640 media was added in all tubes except the stock (T_1). From the stock (T_1), 1 ml of drug was transferred in toT_2 tube. The above was mixed well and 1 ml of the content was transferred to the T_3 tube. This procedure was continued till the last T_{10} tube. From the last tube 1 ml of content was discarded. The final concentration of the drug varied based on the potency. For example, the drug dilution series of *Thuja* Q is represented in Table 1 Clean, dry, sterile U bottomed microtitre plate was taken and 100 µl of each drug dilution were added into each appropriate wells followed by 100 µl of prepared spore suspension. Two separate wells were maintained, one as growth control (100 µl media and 100 µl inoculum) and another as sterility control (100 µl media and 100 µl water). The titrated plates were incubated at 28°C for 48 h in gas-permeable plastic bags as to prevent drying and the results were read. The minimum concentration of antifungal agent that can inhibit the growth of fungal isolates was considered as the MIC of antifungal agents the clinical isolate.

Table 1: Dilutions of *Thuja* 10 M and *Thuja* Q employed for the antifungal susceptibility testing by broth microdilution assay.

Tubes	RPMI 1640 (ml)	Drug dilution (ml)	Final concentration (*Thuja* 10 M)	Final concentration (*Thuja* Q)
Tube 1 T_1	0	2	$1/10^{20,000}$	1/10
Tube 2 T_2	1	1	$0.5/10^{20,000}$	0.5/10
Tube 3 T_3	1	1	$0.25/10^{20,000}$	0.25/10
Tube 4 T_4	1	1	$0.125/10^{20,000}$	0.125/10
Tube 5 T_5	1	1	$0.0625/10^{20,000}$	0.0625/10
Tube 6 T_6	1	1	$0.0312/10^{20,000}$	0.0312/10
Tube 7 T_7	1	1	$0.0156/10^{20,000}$	0.0156/10
Tube 8 T_8	1	1	$0.0078/10^{20,000}$	0.0078/10
Tube 9 T_9	1	1	$0.0039/10^{20,000}$	0.0039/10
Tube 10 T_{10}	1	1	$0.0019/10^{20,000}$	0.0019/10

iii) Minimum Fungicidal Concentrations (MFC)

The wells which had a higher concentration than MIC were taken for the determination of MFC. A loop full of inoculum was taken from the microtitre plates and streaked on to the SDA plates. The plates were incubated at 25°C for 72 h. The concentration having no growth was considered as the MFC value of that particular drug.

RESULTS

The patients attending microbiology laboratory of tertiary eye care hospital in Coimbatore, with suspected keratitis were included in the present study. A total of 35 samples were obtained from the patients suffering from corneal ulcers between the period of October 2012 to January 2013. From these samples, a total of 15 isolates of bacteria and 20 isolates of fungi were obtained. These 20 fungal isolates were used for the further studies. The fungal isolates were subjected to analyses like colony morphology on PDA, KOH and LCB wet mount, for the identification of fungal genera. Out of 35 isolates analysed, *Fusarium* spp. (n=5), *Aspergillus flavus* (n=6), *Bipolaris* spp. (n=3), *Exserohilum* spp. (n=3) and *Curvularia* spp. (n=3) were identified and were designated as *Fusarium* FS1-FS5, *Aspergillus flavus* AS1- AS6, *Bipolaris* BS1-BS3, *Exserohilum* ES1-ES3 and *Curvularia* CS1-CS3, respectively.

Table 2: Percent growth inhibition of *Thuja occidentalis* potencies on ocular fungal isolates (n=20).

Fungal isolates	*Thuja* Q	*Thuja* 30 C	*Thuja* 200 C	*Thuja* 1M	*Thuja* 10M	*Thuja* 50M
BS1	97.71%	88.53%	69.42%	83.94%	83.94%	88.53%
BS2	97.71%	85.47%	74.77%	81.65%	88.53%	87.77%
BS3	97.81%	97.81%	97.81%	97.81%	97.81%	97.81%
ES1	69.17%	74.17%	67.50%	61.67%	82.50%	82.50%
ES2	46.41%	67.97%	75.16%	64.05%	60.78%	57.52%
ES3	45.32%	74.31%	73.65%	66.40%	57.18%	58.50%
CS1	85.99%	75.98%	82.98%	81.98%	73.97%	82.98%
CS2	75.56%	60.00%	74.44%	71.11%	62.22%	74.44%
CS3	46.08%	6.86%	10.78%	0.00%	41.18%	31.37%
FS1	27.44%	19.03%	36.91%	15.88%	19.03%	23.24%
FS2	33.86%	9.49%	17.32%	11.23%	19.93%	28.63%
FS3	37.94%	0.00%	0.00%	0.00%	97.00%	16.92%
FS4	41.49%	1.60%	0.00%	20.21%	0.00%	29.08%
FS5	82.46%	54.39%	61.40%	47.37%	61.40%	70.18%
AS1	37.20%	38.41%	34.78%	61.35%	32.37%	46.86%
AS2	32.14%	58.33%	42.86%	58.33%	47.62%	45.24%
AS3	66.91%	63.61%	71.05%	59.47%	71.05%	61.95%
AS4	59.85%	61.72%	69.19%	71.99%	69.19%	66.39%
AS5	43.94%	66.97%	54.95%	56.96%	47.95%	50.95%
AS6	53.14%	68.17%	64.63%	66.40%	58.44%	62.86%

Table 3: Distribution of the tested ocular fungal isolates (n=20) based on 75%-100% inhibition on treatment with various potencies of homeopathic drug *Thuja occidentalis*

Drug	*Curvularia* spp.	*Exserohilum* spp.	*Bipolaris* spp.	*Fusarium* spp.	*Aspergillus flavus*	No. of isolates inhibited	Isolates %
Thuja Q	CS1,CS2	-	BS1,BS2,BS3	FS5	-	6	30%
Thuja 50M	CS1	ES1	BS1,BS2,BS3	-	-	5	25%
Thuja 30	CS1	-	BS1,BS2,BS3	-	-	4	20%
Thuja 200	CS1	ES2	BS2, BS3	-	-	4	20%
Thuja 1M	CS1	-	BS1,BS2,BS3	-	-	4	20%
Thuja 10M	-	ES1	BS1,BS2,BS3	-	-	4	20%

Table 4: Statistical analysis (Paired sample t test) of *in vitro* activity of various potencies of *Thuja occidentalis* against the ocular fungal isolates (n = 20)

Clinical Fungal Isolate	*Thuja* potencies	*df*	Mean		mean diff	*t stat*	Sig
			Growth control	Treated Plates			
Bipolaris spp.	Q	2	4.43	0.10	-4.33	64.90	**
	30C	2	4.43	0.41	-4.02	17.80	**
	200C	2	4.43	0.84	-3.58	8.07	**
	1M	2	4.43	0.53	-3.89	13.67	**
	10M	2	4.43	0.43	-3.99	16.61	**
	50M	2	4.43	0.38	-4.05	19.68	**
Exserohilum spp.	Q	2	4.72	2.24	-2.48	16.83	**
	30C	2	4.72	1.32	-3.40	14.67	**
	200C	2	4.72	1.30	-3.42	9.46	**
	1M	2	4.72	1.69	-3.03	10.69	**
	10M	2	4.72	1.62	-3.10	26.39	**
	50M	2	4.72	1.66	-3.06	25.96	**
Curvularia spp.	Q	2	3.24	1.01	-2.23	5.96	**
	30C	2	3.24	1.72	-1.52	2.25	ns
	200C	2	3.24	1.46	-1.79	2.46	ns
	1M	2	3.24	1.67	-1.58	1.81	ns
	10M	2	3.24	1.33	-1.91	6.21	**
	50M	2	3.24	1.22	-2.02	4.03	**
Fusarium spp.	Q	4	3.20	1.89	-1.31	10.29	**
	30C	4	3.20	2.82	-0.38	1.80	ns
	200C	4	3.20	2.62	-0.58	2.10	ns
	1M	4	3.20	2.75	-0.45	2.10	ns
	10M	4	2.05	2.66	0.61	-2.79	ns
	50M	4	3.20	2.23	-0.96	6.97	**
Aspergillus flavus	Q	5	3.38	1.67	-1.70	5.99	**
	30C	5	3.38	1.33	-2.04	8.48	**
	200C	5	3.38	1.42	-1.96	6.31	**
	1M	5	3.38	1.26	-2.12	12.25	**
	10M	5	3.38	1.48	-1.89	6.23	**
	50M	5	3.38	1.46	-1.92	8.28	**

Values are the means of triplicates of Radial diameter (cm) of growth control & treated plates on day 3, 4 and 5. Paired t test analysis. Note: * p<.05, ** p <.01, ns not significant; Critical t @ df(2)=2.92 one tailed p <.05; Critical t @ df (4)=2.13 one tailed p <.05; Critical t @ df(5)=2.02 one tailed p <.05

Plate assay results for the determination of antifungal activity of *Thuja* showed that the drug could inhibit the growth of fungal isolates. All the potencies of *Thuja* had good inhibitory activity against *Bipolaris* spp., followed by *Curvularia* spp., *Exserohilum* spp. and *A. flavus. Thuja* Q, particularly exhibited inhibition in the range of 75% -100% among 30% of the isolates tested. All the potencies of *Thuja* showed the highest inhibition percentage of 97.81 against *Bipolaris* isolate BS3. *Thuja* 30, 200 and 1M could not inhibit (0%) the *Fusarium* isolate FS3. Similarly, nil inhibition was observed for *Thuja* 200 & *Thuja* 10M for *Fusarium* isolate FS4 and *Thuja* 1M for *Curvularia* isolate CS 3. Among *Exserohilum* isolates, the maximum inhibition (82.5 %) was noted in ES 1 by *Thuja* 10 M and 50 M. *Thuja* Q, 200 and 50 M exhibited maximal inhibition of 85.99 %, 82.98 % and 82.98 % respectively for the *Curvularia* isolate CS 1. Among fusaria, maximal inhibition of 97% was exhibited by *Thuja* 10 M for the isolate FS3 followed by *Thuja* Q (82.46%) for the isolate FS5. With respect to *A. flavus*, the potencies of 10M could inhibit AS4 (71.99%) and AS3 (71.05%) maximally (Table 2 and 3). Various potencies of *Thuja* exhibited poor inhibition (<25%) on majority of the *Fusarium* isolates (FS1, FS2, FS3 & FS4) and also on the *Curvularia* isolate CS3. Statistical analysis utilising paired t test revealed significant inhibition of all the test isolates belonging to various fungal genera by *Thuja* Q and 50 M. Significant growth inhibition was exhibited by *Thuja* 30 C, 200 C, I M for *Bipolaris*, *Exserohilum* & *A. flavus* isolates and *Thuja* 10 M for all the isolates tested except fusaria. Nil inhibition with *Thuja* potencies was observed as follows: *Thuja* 10M on FS4, *Thuja* 30 on FS 3, *Thuja* 200 on FS3 and FS4 and *Thuja* IM on FS3 and CS3 (Table 4).

Thuja 10M showed an MIC of $0.125/10^{20,000}$ and MFC of $0.25/10^{20,000}$ for all *Bipolaris* isolates *i.e*., BS1, BS2 and BS3. *Thuja* Q showed an MIC of 0.0625/10 and MFC of 0.125/10 for all *Bipolaris* isolates (Table 5).

Table 5: MIC and MFC of *Thuja* 10 M and *Thuja* Q for *Bipolaris* isolates

Drug	MIC	MFC
Thuja 10 M	$0.125/10^{20,000}$ (4^{th} dilution)	0.25/10 (3^{rd} dilution)
Thuja Q	0.0625/10 (5^{th} dilution)	0.125/10 (4^{th} dilution)

DISCUSSION

The incidence of fungal keratitis, risk factors, and the type of agent causing may vary among different geographic regions [5, 15 & 16]. Worldwide, the reported incidence of fungal keratitis is 17% to 36% [16 - 18], whereas in India, it is 44% to 47% [5, 20-22]. The outcome of mycotic keratitis depends ultimately on the interplay of the agent (virulence, resistance to drugs, and toxicity) and host factors (predisposing factors, inflammatory response, and hypersensitivity reactions) in addition to timely diagnosis and appropriate medical treatment. In the present investigation, the common aetiological agent for corneal ulcer was identified as fungi rather than bacteria. The similar results were obtained by Bharathi *et al*. [1] in south India. Leck *et al*. [3] also reported similar results from the south, north and east India. But other studies from Thailand and Malaysia reported that the most frequent causative agent of microbiological keratitis was bacteria [23&24]. This revealed that in India fungal keratitis is more prevalent than bacterial keratitis. Mycotic keratitis is expected to be more common in tropical and subtropical locations than in the temperate regions. A hot, humid climate and an agriculture-based occupation of a large population make fungal keratitis more frequently in tropical countries. Gopinathan *et al*. [25] made the similar observation. In the present study a total of 20 fungal isolates *viz*., *Fusarium* spp (n=5), *Aspergillus flavus* (n=6), *Bipolaris* spp. (n=3), *Exserohilum* spp. (n=3), and *Curvularia* spp (n=3) were identified. The isolates were confirmed by microscopic and macroscopic observations. Srinivasan *et al*. [5] revealed that out of 155 fungal isolates cultured from 154 corneal ulcers 47.1% were *Fusarium* spp. 16.1% were *Aspergillus* spp. and the remaining organisms were a diverse mixture of unusual fungal pathogens including a large number of unidentified dematiacious fungal species (13.5%) and hyaline fungal species (9.6%). The pattern of fungal organisms, dominated by *Fusarium* spp. is similar to the spectrum of microbial keratitis reported from south Florida by Liesegang and Forster [4] and from Ghana by Hagan *et al.* [26]. By contrast, in most of the world *Aspergillus* spp. or *Candida* spp. are the predominant fungal pathogens responsible for mycotic keratitis (Jones 1975) [15]. In the temperate climate of Nepal, Upadhyay *et al.* [18] found that *Aspergillus* spp. accounted for 47% of all fungal pathogens followed by *Candida* spp. (13.2%) and *Fusarium* spp. (11.7%).

Owing to the undesirable side effects of the conventional drug of choice used for the treatment of mycotic keratitis and also, the emergence of antifungal resistant strains, the present study was carried out with an idea of analysing the potential use of homeopathic drugs in the control of molds involved in mycotic keratitis. Clausen *et al*. [27] has reported that, pathogenetic, prophylactic and therapeutic study designs seem to be equally suited for homeopathy basic research using high dilutions. Homeopathy proves to be the one of the best alternative treatment being economical, minimal to no side-effects claimed so far, and no known contraindications or drug interactions reported so far when used along with conventional medications. In the present study, all the potencies of *Thuja* had good inhibitory activity against *Bipolaris* spp., followed by *Curvularia* spp., *Exserohilum* spp. and *A. flavus.* The antifungal effect of homeopathy drugs has been reported by many workers. *In vitro* testing of antifungal effectiveness of Sulphur iodatum 1M and Petroleum 30 against the cellulolytic fungi *Aspergillus niger* was first reported by Garg [28]. Dua and Atri *et al*. [29] has reported fungitoxic effect of the drug Lycopodium against *Alternaria solani.*

The homoeopathic drugs *Thuja* and Natrum muriaticum were found to be effective on *Fusarium* spp. as reported by Hussain *et al.* [30]. Khanna and Chandra [31] investigated the control of tomato fruit rot caused by *Fusarium roseus* with homoeopathic drugs Kali iodatum (149CH) and *Thuja occidentalis* (87CH), in pre and post-harvest conditions and obtained significant results. Gupta *et al*. [32] has reported that the methanolic extract of *Thuja* has good activity against clinically isolated fungi. Jahan *et al.* [33] described the antifungal activity of *T. occidentalis* extracts against *Saccaromyces cereviciae, A. parasiticus, Trichophyton rubrum, Macrophomina, F. solani and Candida albicans.* Gupta *et al*. [34] has stated that *Thuja* 30, 200 and 10M are very effective against pathogenic isolate *Curvularia lunata,* however other dilutions are not much effective. *Thuja* Q, 30, 200 are found highly potent against *A. flavus* and 50M against *A. niger* as detailed by Srivastava [35]. The pre-treated and the post-treated data of the mean of the radial growth (cm) of ocular fungal isolates of various genera tested in the plate assay method were subjected to statistical analysis employing paired- t- tests. Most of the potencies used in the plate assay method, showed statistically significant inhibition. As varying potencies of the drug *Thuja* showed good inhibitory activity against *Bipolaris* spp. MIC and MFC analyses were carried out with the selected drugs *viz*., *Thuja* 10 M and *Thuja* Q against the *Bipolaris* isolates BS1, BS2 and BS3. *Thuja* 10M showed an MIC of $0.125/10^{20,000}$ and an MFC of $0.25/10^{20,000}$whereas *Thuja* Q showed an MIC of 0.0625/10and an MFC of 0.125/10 for all *Bipolaris* isolates. Such MIC/MFC reports of *in vitro* activity of highly diluted-dynamized homeopathic solutions are rare.

CONCLUSION

The mode of action of the homeopathic drug *Thuja* is not evaluated in this study. The detailed analysis of the variation in the efficacy of different potencies of same homeopathic medicine in antifungal susceptibility testing is a subject of further study. It is highly imperative to state that the importance of *in vitro* studies lies in the fact that it possible to obtain preliminary evidence on the effect of high dilutions under controlled laboratory conditions, thereby testing on the main principles of homeopathy *viz*., similarity in the mode of action at the cellular level and the effect of drug dilution on the drug activity, here with special emphasis on antifungal susceptibility testing. Furthermore, the *in vitro* studies can also be utilised for finding the exact molecular targets of homeopathic agents. The present investigation concludes that homeopathic drugs have significant inhibitory activity against the fungi causing keratitis, irrespective of the potencies.

A good inhibition (75% - 100%) of *Thuja* (Q, 30, 1 M, 10 M & 50 M) was found among all the three isolates of *Bipolaris* subjected for analyses. Further, out of 5 different fungal genera tested, *Thuja* potencies *viz*., Q, 200 & 50 M were effective against 3 fungal genera. The follow up antifungal susceptibility analyses by broth microdilution utilising *Thuja* 10 M and *Thuja* Q revealed that both the potencies had inhibitory and fungicidal activity against *Bipolaris* isolates. It is evident that no definite co-relation exists between various potencies of the same homeopathic drug with regard to their antimycotic properties.

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